TRADE SECRET

Study Title

Cross-Species Comparison of FRD-902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing

AUTHOR: Shawn A. Gannon, B.S.

ORIGINAL REPORT COMPLETED: December 8, 2008

REPORT REVISION 1 COMPLETED: February 2, 2009

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-17751-1579

WORK REQUEST NUMBER: 17751

SERVICE CODE NUMBER: 1579

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

This is an electronic version of the final report. No signatures are necessary.

REASON FOR REVISION 1

In the original report, the test substance was referred to by the Haskell identification number of the dose material, the Haskell identification number of the analytical standard material, or the compound name. To eliminate confusion, all Haskell identification number references were changed to compound name.

SUMMARY

The objectives of this study were to evaluate the clearance of FRD-902 in male and female cynomolgus primates and Sprague-Dawley rats following intravenous dosing, and to conduct a cross-species comparison of FRD-902 plasma elimination kinetics.

Rats (3 males and 3 females per dose level) received a single 10 mg/kg or 50 mg/kg intravenous bolus of FRD-902 prepared in sterile phosphate buffered saline. Six non-naïve Cynomolgus monkeys (3 male and 3 female) received a single 10 mg/kg intravenous bolus of FRD-902 formulated in the same manner as the rat dose solution. Blood was collected at multiple time points over 7 days (rat) or 21 days (primate) and the plasma concentration of FRD-902 was determined.

FRD-902 was rapidly eliminated in primates and rats following a single intravenous dose. Clearance times were similar (less than 12 hours) among male and female primates and female rats, while slightly longer clearance times (approximately 22 hours) were observed in male rats. The results in rats dosed intravenously with FRD-902 were similar to those observed previously in rats following a single oral dose.

Based on the results of a single dose oral study in mice, this species clears FRD-902 less rapidly than either the primate or the rat. Clearance times in mice following oral dosing were 140 and 60 hours in males and females, respectively. Thus, among the 3 species evaluated, the plasma clearance of FRD-902 is most similar and more rapid in primates and rats, with mice having a comparatively longer clearance time.

INTRODUCTION

The objectives of this study were to evaluate the clearance of FRD-902 in male and female cynomolgus primates and Sprague-Dawley rats following intravenous dosing, and to conduct a cross-species comparison of FRD-902 plasma elimination kinetics. Previous pharmacokinetic studies in rats[(1)] and mice[(2)] dosed orally with FRD-902 showed that clearance was more rapid in the rat than in the mouse. In both rodent species, clearance was more rapid in females compared to males. The following study further characterizes the comparative elimination of FRD-902 to include primates.

MATERIALS AND METHODS

A. Test Substance

FRD-902 was received at DuPont Haskell as an 82.6% concentrated aqueous solution and assigned Haskell identification number 28072. Dose solutions were prepared by diluting this compound to the appropriate concentration in a physiological buffer acceptable for intravenous dosing.

B. In-life Phase

1. Rat

A total of 6 Crl:CD SD rats (3 males and 3 females) per dose level were assigned to the study. The animals were fasted overnight prior to dosing and through the first 2 hours of blood collection. The low dose group received a single 10 mg/kg intravenous bolus of FRD-902 formulated in sterile phosphate buffered saline, pH 7.6 at a dose volume of 1 ml/kg. The high dose group received a single 50 mg/kg dose of FRD-902 formulated in the same manner as the low dose. Blood (approximately 0.1 ml per sample) was collected from the tail vein at predose and approximately 0.083 (5 min), 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8, 12, and 24 hours postdose. Additional blood samples were collected once daily on test days 2-7. Samples were centrifuged to generate plasma that was subsequently analyzed at DuPont Haskell.

2. Primate

The details for the in-life phase of the primate study are included in Appendix A. Briefly, a total of 6 non-naïve Cynomolgus monkeys (3 male and 3 female) were assigned to the study. The animals were fasted overnight prior to dosing and through the first 4 hours of blood sample collection. All primates received a single 10 mg/kg intravenous bolus of FRD-902 formulated in sterile phosphate buffered saline, pH 7.6 at a dose volume of 2 mL/kg. Blood (approximately 0.5 mL per sample) was collected from the femoral vessel at predose and at approximately 0.083 (5 min), 0.167 (10 min), 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8, 12, and 24 hours postdose.

Additional blood samples were collected once daily on test days 3-21. Samples were centrifuged to generate plasma that was shipped to DuPont Haskell for analysis.

C. Plasma Sample Analyses

1. Rat

The plasma samples were received and stored frozen prior to laboratory use. The samples were prepared for analysis by pipeting 300 µL acetonitrile into a 1.5 mL microcentrifuge tube, and pipeting 100 µL of plasma sample. The sample tubes were then vortexed for 1 minute and centrifuged at 14,000 RCF for 30 minutes. After centrifugation, 200 µL of sample supernatant was placed into a HPLC vial and 800 µL of HPLC grade water was added and mixed. As necessary, additional sample dilutions were performed using the 15% acetonitrile in HPLC grade water solvent to ensure that the sample responses were within the calibration curve. For plasma samples that were diluted 20x and internal standard of PFOA was used to negate potential matrix effects. The limit of quantitation (LOQ) for the study samples was 1 ng/mL, which was defined as the lowest calibration standard concentration multiplied by the sample preparation factor of 20x. The lowest calibration standard at 0.05 ng/mL had at least a 5x blank response and acceptable calibration curve accuracies within 80-120%.

The prepared samples were analyzed by LC/MS using the following parameters:

HPLC Instrument:	Agilent Model 1200
MS Instrument:	Applied Biosystems API 4000
LC Parameters:	
Column:	Zorbax RX-C8; 2.150 x 4.6 mm with 5 micron particle size
Mobile Phase:	A: 0.15% formic acid in HPLC grade water B: 0.15% formic acid in acetonitrile
Column Temperature:	35ºC
Injection Volume:	100.0 µL or 10 µL
MS Parameters:	
Ion Source:	Turbo Spray, Negative Ion
Temperature (TEM):	300
Dwell:	200 msec
Curtain Gas Flow (CUR):	25.0
GS1:	50
GS2:	50
IonSpray (IS) Voltage:	-3500
CAD:	10.0
EP:	-10.0
Quadrupole Resolution:	Quad. 1: Unit Quad. 3: Unit

MRM Settings:	Q1 Mass	Q3 Mass	DP	CE	CXP

FRD-902 1st Transition:	328.00	285.00	-15.00	-6.00	-7.00
FRD-902 2nd Transition:	285.00	169.00	-40.00	-10.00	-3.00
	Responses of 1st and 2nd transitions added together for analysis				
Internal Standard Trans.:	417.00	372.00	-30.00	-15.00	-11.00

HPLC Mobile Phase Gradient:

Step	Total Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0	0.00	400	40.0	60.0
1	6.70	400	40.0	60.0

2. Primate

The plasma samples were received and stored frozen prior to laboratory use. The samples were prepared for analysis by pipeting 100 µL of plasma sample into 1.7 mL centrifuge tubes. Next, a pipet was used to add 300 µL acetonitrile. The sample tubes were vortexed briefly to mix homogenously. The samples were then centrifuged at 14,000 RCF for 10 minutes at 20ºC. After centrifugation, 800 µL of HPLC grade water and 200 µL of sample supernatant were placed into HPLC vials and mixed. As necessary, additional sample dilutions were performed using a dilution solvent (15% acetonitrile in HPLC grade water) to ensure that the sample responses were within the calibration curve.

Prior to LC/MS/MS analysis a ^{13}C-PFOA internal standard was added to all prepared samples, diluted samples, calibration standards, and fortification QC samples to correct for possible matrix effects. The limit of quantitation (LOQ) for the study samples was 1 ng/mL, which was defined as the lowest calibration standard concentration multiplied by the sample preparation factor of 20x. The lowest calibration standard at 0.05 ng/mL had at least a 5x blank response and acceptable calibration curve accuracies within 80-120%.

The prepared samples were analyzed by LC/MS using the following parameters:

HPLC Instrument:	Agilent Model 1100
MS Instrument:	Applied Biosystems API 4000
LC Parameters:	
Column:	Analytical: Zorbax RX-C8; 2.1x150 mm with 5 micron particle size Delay: Zorbax SB-C18 2.1x150 5 um particle size
Mobile Phase:	A: 0.15% acetic acid in HPLC grade water B: 0.15% acetic acid in acetonitrile
Column Temperature:	35ºC
Injection Volume:	40.0 µL
MS Parameters:	
Ion Source:	Turbo Spray, Negative Ion
Temperature (TEM):	300

Dwell:	200 msec
Curtain Gas Flow (CUR):	20.0
GS1:	30
GS2:	30
IonSpray (IS) Voltage:	-3500
CAD:	10.0
EP:	-10.0
Quadrupole Resolution:	Quad. 1: Unit
	Quad. 3: Unit

MRM Settings:	Q1 Mass	Q3 Mass	DP	CE	CXP
FRD-902 1st Transition:	328.00	285.00	-15.00	-6.00	-7.00
FRD-902 2nd Transition:	285.00	169.00	-40.00	-10.00	-3.00
	Responses of 1st and 2nd transitions added together for analysis				
Internal Standard Trans.:	415.00	370.00	-30.00	-15.00	-11.00

HPLC Mobile Phase Gradient:

Step	Total Time (min)	Flow Rate (µL/min)	A (%)	B (%)
0	0.00	400	90.0	10.0
1	1.00	400	90.0	10.0
2	1.10	400	30.0	70.0
3	7.00	400	30.0	70.0
4	7.10	400	90.0	10.0
5	13.0	400	90.0	10.0

RESULTS AND DISCUSSION

The average plasma concentrations for rat and primate are reported in Table 1. The individual data are reported in Appendix C (rat) and D (primate). The plasma concentration data was plotted against time (Figures 1-2, rat; Figure 3 primate) and clearance time was determined (Table 2).

FRD-902 was rapidly eliminated in primates and rats following a single intravenous dose (Tables 2-3). Clearance times were similar (less than 12 hours) among male and female primates and female rats, while slightly longer clearance times (approximately 22 hours) were observed in male rats. The results in rats dosed intravenously with FRD were similar to those observed in rats following a single oral dose in a previous study.(1)

The high level of sensitivity and extended evaluation of the plasma elimination kinetics distinguish this work form less robust studies that may employ higher analytical detection limits and/or shorter evaluation times. Less robust elimination kinetics studies may produce misleading linearity over the initial elimination phase and introduce uncertainty into any subsequent kinetic modeling of the elimination kinetics (Figures 3-4). The data generated in this study provided a basis for cross-species comparisons of FRD-902 plasma elimination kinetics.

Based on the results of a single dose oral study, mice clear FRD-902 less rapidly than either the primate or the rat.(2) Clearance times in mice following oral dosing were 140 and 60 hours in males and females, respectively. Thus, among the 3 species evaluated, the plasma clearance of FRD-902 is most similar and more rapid in primates and rats, with mice have a comparatively longer clearance time.

REFERENCES

1. DuPont Haskell (2008). HFPO Dimer Acid Ammonium Salt: Biopersistence and Pharmacokinetic Screen in the Rat. Unpublished report, DuPont-24281.

2. DuPont Haskell (2008). FRD-902: Biopersistence and Pharmacokinetic Screen in the Rat. Unpublished report, DuPont-25300.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

LOQ = limit of quantitation
NA = not applicable
SD = standard deviation

Table 1
Average plasma concentrations of FRD-902 in primate and rat following intravenous dosing

Time Point	10 mg/kg Primate				10 mg/kg Rat				50 mg/kg Rat			
	Male		Female		Male		Female		Male		Female	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
0	<LOQ	NA	<LOQ	NA	15	19	11	14	9	7	21	2
5 min	195,000	20664	222333	78034	78800	38438	62867	9963	377667	27610	363000	48218
10 min	178,333	14364	212667	36199	-	-	-	-	-	-	-	-
15 min	160,000	19313	190333	37287	56433	4957	31900	917	345000	43966	275000	6557
30 min	106,700	34892	149000	21656	52500	3869	19767	404	285333	32316	190500	21254
1 Hr	78,900	16691	100467	14624	45733	6637	6427	863	233333	30989	108967	13058
2 Hr	44,067	7306	50733	6034	42833	1815	2077	622	162333	32655	33967	4821
4 Hr	16,467	5552	16167	1069	20933	3239	336	61	58933	21658	4327	460
8 Hr	5407	2785	4917	857	6703	1661	269	233	19433	13530	842	767
12 Hr	1951	1308	1413	251	3733	1257	75	19	8923	5312	685	593
24 Hr	246	184	83	52	776	230	7	4	1884	1556	78	45
48 Hr	-	-	-	-	75	30	16	15	1020	287	84	42
72 Hr	67	50	28	26	44	7	12	3	147	48	101	51
96 Hr	15	9	3	2	56	32	6	3	127	60	37	22
120 Hr	8	2	3	2	41	24	2	NA	112	67	45	23
144 Hr	8	1	9	6	39	23	2	0.3	89	48	52	16
168 Hr	4	1	1	NA	22	5	2	NA	76	13	26	4
192 Hr	3	1	1	NA	-	-	-	-	-	-	-	-
216 Hr	4	1	<LOQ	NA	-	-	-	-	-	-	-	-
240 Hr	4	2	1	NA	-	-	-	-	-	-	-	-
264 Hr	5	1	3	0.4	-	-	-	-	-	-	-	-
288 Hr	3	1	1	NA	-	-	-	-	-	-	-	-
312 Hr	3	1	<LOQ	NA	-	-	-	-	-	-	-	-
336 Hr	3	1	1	NA	-	-	-	-	-	-	-	-
360 Hr	2	1	2	NA	-	-	-	-	-	-	-	-
384 Hr	2	0.4	2	NA	-	-	-	-	-	-	-	-
408 Hr	<LOQ	NA	<LOQ	NA	-	-	-	-	-	-	-	-
432 Hr	<LOQ	NA	<LOQ	NA	-	-	-	-	-	-	-	-
456 Hr	<LOQ	NA	<LOQ	NA	-	-	-	-	-	-	-	-
480 Hr	<LOQ	NA	<LOQ	NA	-	-	-	-	-	-	-	-
504 Hr	<LOQ	NA	<LOQ	NA	-	-	-	-	-	-	-	-

Table 2
Clearance time (hr) in primate and rat following intravenous dosing

	Primate	Rat	
	10 mg/kg	10 mg/kg	50 mg/kg
Male	11	22	17
Female	10	3	4

Note: Clearance time is commonly defined as the time it takes to eliminate effectively all (98.4%)of the administered test substance.

Table 3
Half-life of FRD-902 in primate plasma over the time interval corresponding to clearance time

	Time Interval (hr)	Lambda (hr^{-1})	Half-life (hr)	Regression r^2
Male	0-12	0.3845	1.8	0.9556
	4-12	0.2666	2.6	0.9930
Female	0-12	0.4288	1.6	0.9663
	4-12	0.3047	2.3	0.9998

FIGURES

Figure 1
FRD-902 plasma concentration in male and female rats following a 10 mg/kg intravenous dose

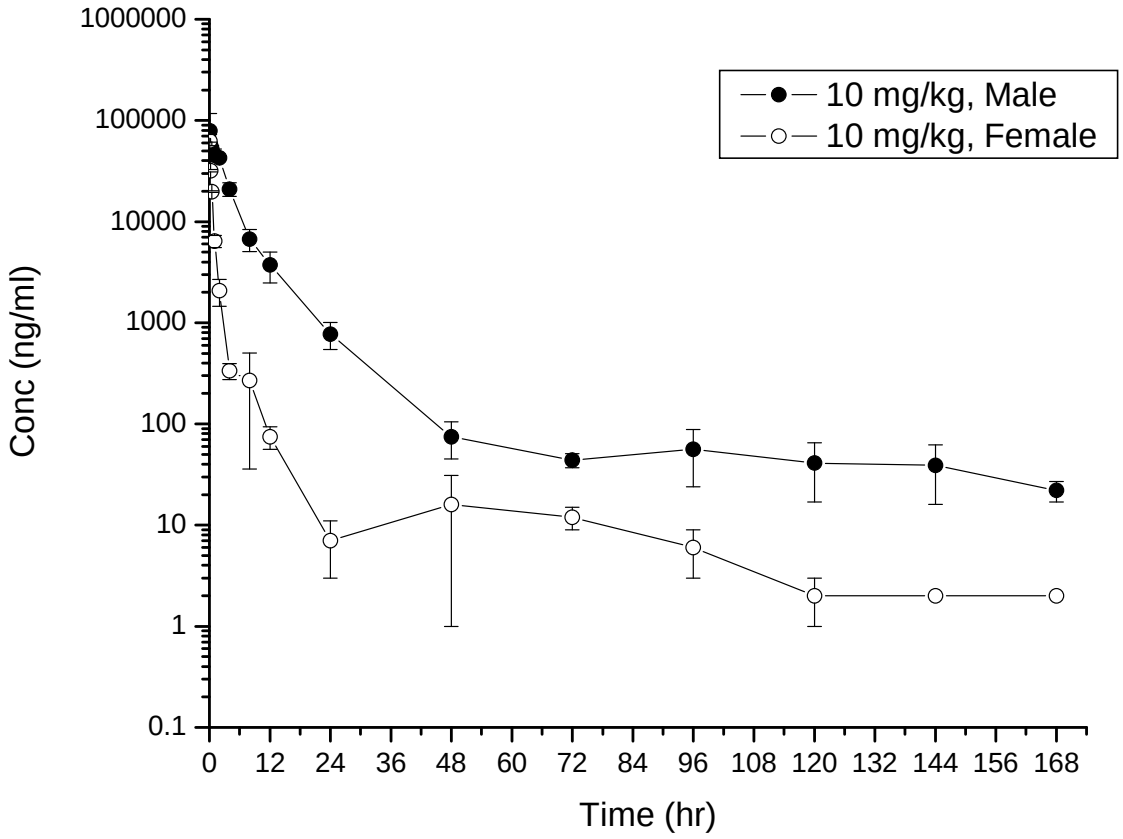


Figure 2
FRD-902 plasma concentration in male and female rats following a 50 mg/kg intravenous dose

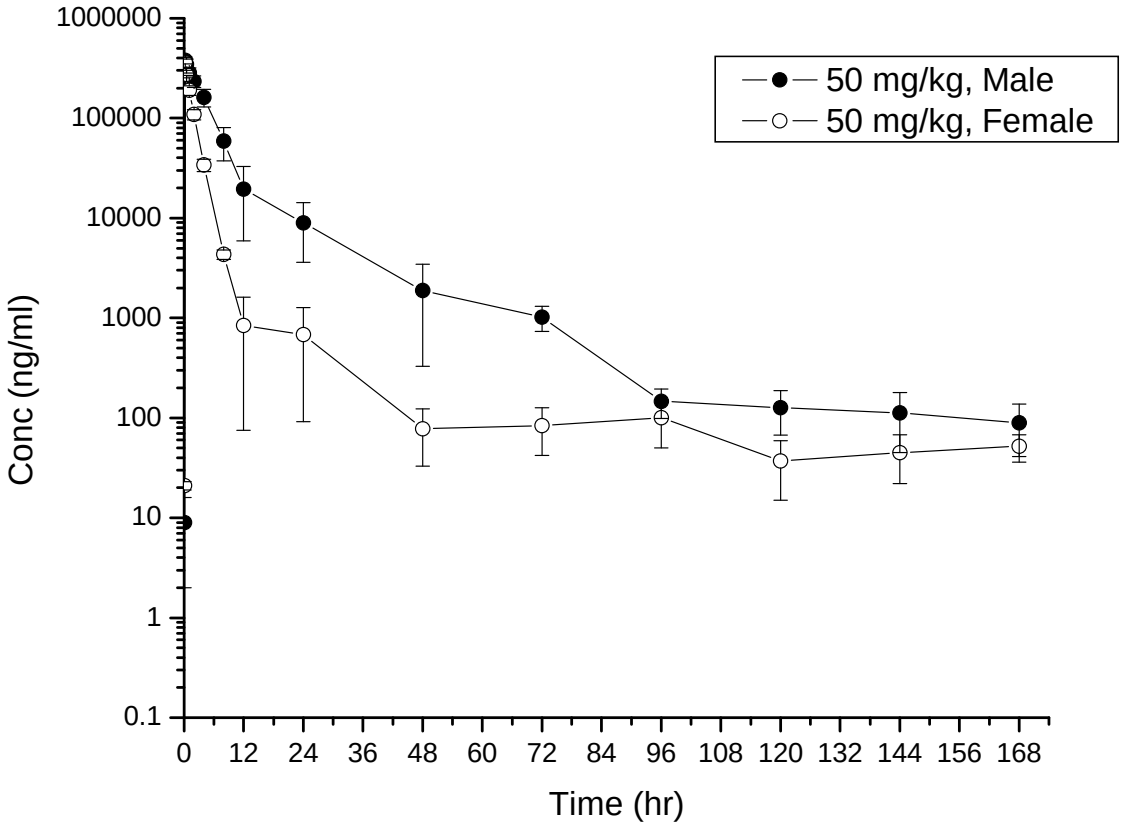


Figure 3
FRD-902 plasma concentration in male and female primates following a 10 mg/kg intravenous dose

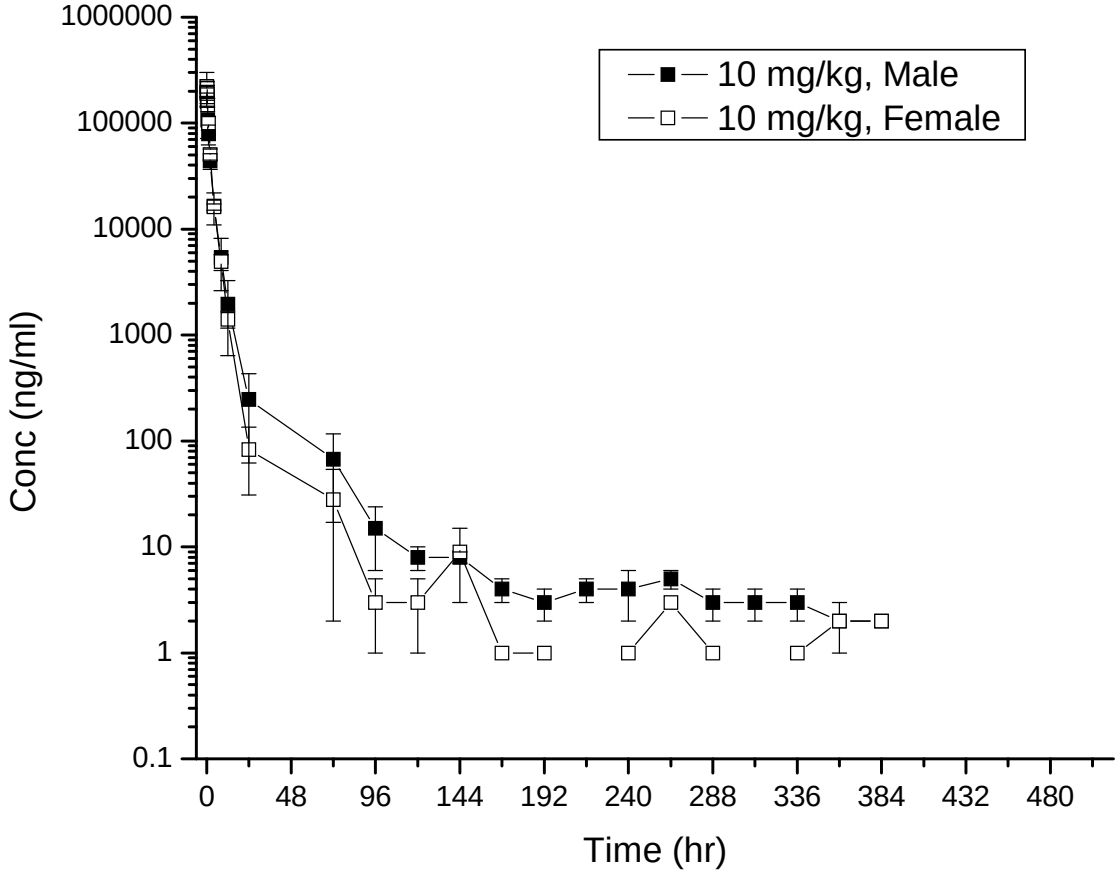
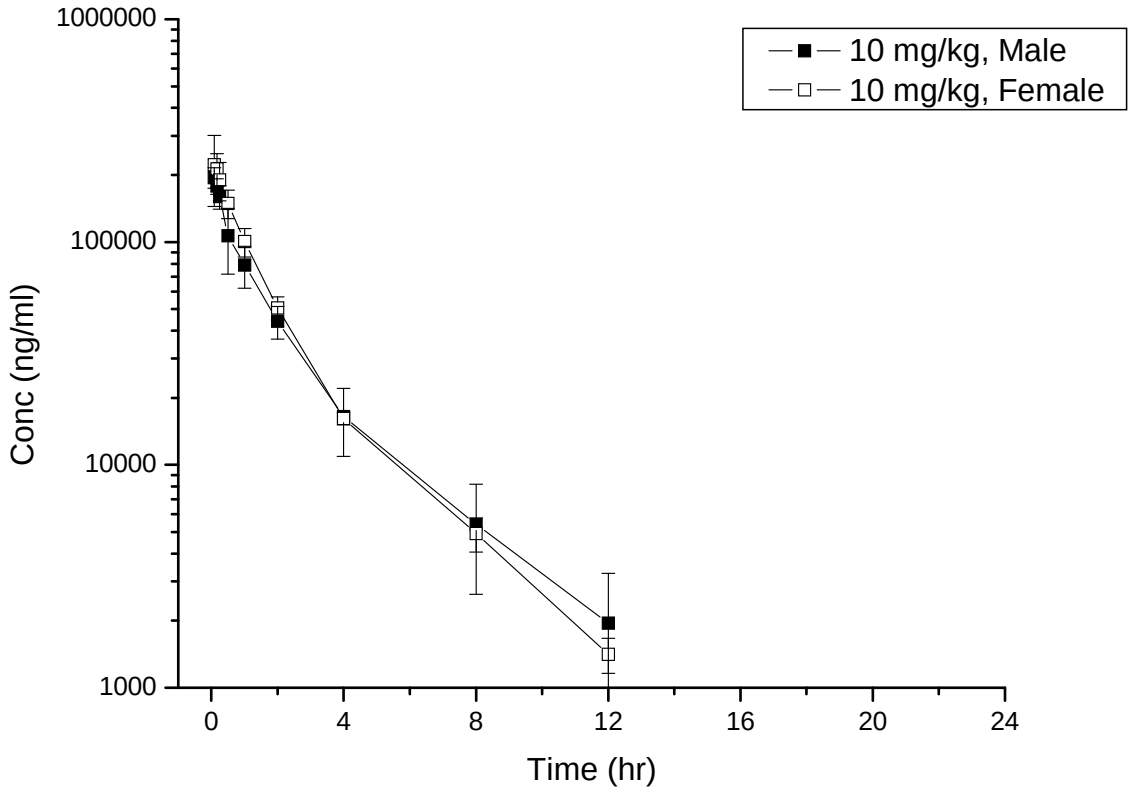


Figure 4
FRD-902 plasma concentration from zero to 12 hours in male and female monkeys following a 10 mg/kg intravenous dose



Appendix A
Protocol for Primate Study

MPI RESEARCH

TITLE OF STUDY: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose

TEST ARTICLE: FRD-902

TESTING FACILITY: MPI Research, Inc., 54943 N. Main Street, Mattawan, MI 49071-9399

MPI RESEARCH STUDY NUMBER: 125-099

SPONSOR STUDY NUMBER: WR 17751, SC 1579

STUDY DIRECTOR: Travis L. Devlin, M.S., L.A.T.
PHONE: (269) 668-3336 ext. 1707 **FAX:** (269) 668-4151 **E-MAIL:** travis.devlin@mpiresearch.com

MPI RESEARCH STUDY DIRECTOR APPROVAL*: _____ Date: 7-29-08
Travis L. Devlin, M.S., L.A.T.

SPONSOR: DuPont Haskell Global Centers for Health and Environmental Sciences
Stine-Haskell Research Center
1090 Elkton Road
Newark, DE 19714

SPONSOR REPRESENTATIVE(S)/DESIGNEE(S): Shawn A. Gannon, B.S.
PHONE: (302) 451-3396 **FAX:** (302) 451-3568 **E-MAIL:** shawn.a.gannon@usa.dupont.com

AGENCY SUBMISSION: ☒ May be submitted to the U.S. Environmental Protection Agency (EPA)
☐ Will not be submitted to any reviewing agency

GOOD LABORATORY PRACTICE GUIDELINES: This nonclinical laboratory study is not intended to be conducted in full accordance with the United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations, 21 CFR Part 58, but will be conducted in accordance with MPI Research Standard Operating Procedures (SOPs).

QUALITY ASSURANCE: No QA inspections; non-GLP study

ANIMAL WELFARE: Animal welfare for this study will be in compliance with the U.S. Department of Agriculture's (USDA) Animal Welfare Act (9 CFR Parts 1, 2 and 3). The Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Academy Press, Washington, D.C., 1996, will be followed. This facility maintains an Animal Welfare Assurance statement with the National Institutes of Health Office of Laboratory Animal Welfare. In order to ensure compliance, this protocol will be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) before the initiation of treatment. The Sponsor representative/designee, by his or her signature, attests that the activities specified in this protocol do not unnecessarily duplicate any previous experiment. No procedures are anticipated to be used, or test article effects seen, which would cause more than momentary pain or distress to the animals. The acute oral dose in rodents for this compound is greater than 1000 mg/kg. Preliminary work in rats dosed intravenously at the dose level selected for this protocol and at a higher dose level suggests that there will be no adverse affects. Should severe test article effects be observed, the Clinical Medicine Department staff will be notified.

* The Study Director's signature date is the date of initiation of this study. Dosing begins as soon as possible thereafter.

APPROVED

Title of Study: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose
MPI Research Study Number: 125-099
Sponsor Study Number: WR 17751, SC 1579

ARCHIVES: All raw data, documentation, records, protocol, reserve samples (if applicable), specimens (if applicable), and the final report generated as a result of this study will be retained at MPI Research, or an approved archive facility contracted by MPI Research, for a period of 1 year following completion of the study (final report issue date). Retention of materials after the times stated above will be subject to future contractual agreements between the Sponsor and MPI Research.

REPORT: After completion of the study, an unaudited draft report (MPI Research PK format) containing the results of all tests, analyses, observations and measurements required by this protocol will be submitted to the Sponsor representative/designee. After receipt of any Sponsor comments, the final signed report will be issued. Six months after issuance of the draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, the draft report may be issued as a final report, signed by the Study Director, and submitted to the Sponsor.

Brief Description:

A total of 6 non-naïve cynomolgus monkeys (3 males and 3 females) will be initially assigned to study. The animals will be fasted overnight prior to dosing and through the first 4 hours of blood sample collection (total fasting time not to exceed 24 hours).

Test Article Administration
All animals will receive a single intravenous (IV) bolus dose of the appropriate test article formulation in a peripheral vein, as outlined in the study design table below. If a catheter is used for dosing, the catheter will be flushed with approximately 1 mL of sterile 0.9% Sodium Chloride for Injection, USP following dosing. Unless otherwise indicated, intravenous doses will be administered via bolus injection.

Group	Test Article	Number of Males/Females	Dose Route	Vehicle	Dose Level (mg/kg)	Dose Volume (mL/kg)	Matrix Collected
1	FRD-902	3/3	IV	[A]	10	2	Blood[B]

[A] Sterile Phosphate Buffered Saline (final formulation pH = 7.6)
[B] Blood samples will be collected predose and at approximately 0.083 (5 min), 0.167 (10 min), 0.25 (15 min.), 0.5 (30 min.), 1, 2, 4, 8, 12, and 24 hours postdose. Additional blood samples will be collected once daily on Days 3-21.

Pharmacokinetic Blood Collection
Blood samples (approximately 0.5 mL/sample) will be collected from the femoral vessels at the time points specified in the study design table above and placed into tubes containing K_2EDTA. All blood samples will be placed on an ice block (or wet ice) following collection. The samples will be centrifuged and the resulting plasma will be separated and stored frozen at approximately -70°C until shipped on dry ice to Stine-Haskell Research Center, Newark, Delaware, for analysis (following separation, the plasma may be initially placed on dry ice prior to being stored in the -70°C freezer).

Sample Information: All plasma samples will be labeled with the MPI Research study number, animal number, group number/sex, matrix, and the date and time interval of collection.

Title of Study: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose
MPI Research Study Number: 125-099
Sponsor Study Number: WR 17751, SC 1579

Shipping Instructions: Plasma samples will be shipped on dry ice to the address below via overnight, weekday delivery. Unless indicated otherwise, samples will be shipped on the first Monday, Tuesday, Wednesday, or Thursday following collection of the samples on Days 7, 14, and 21.

Primary Contact for Shipment of Plasma Samples
DuPont Haskell Global Centers for Health and Environmental Sciences Attn: Michael Mawn, Ph.D. Stine-Haskell Research Center 1090 Elkton Road Newark, DE 19714 Telephone: (302) 451-3365 Telefax: (302) 451-3571 E-mail: michael.p.mawn@usa.dupont.com

Bioanalytical analysis of the plasma samples will be conducted independently by the Sponsor or a Sponsor-designated laboratory and the results will not be included as an appendix to the final report issued by MPI Research. The Sponsor and/or Sponsor-designated laboratory will be responsible for the conduct, reporting, and any regulatory requirements for these analyses.

Test Article Preparation Instructions

Vehicle Sampling Procedure: Prior to preparation of the doing formulation, a single 10.0 mL sample of the vehicle to be used to prepare the dosing formulation (Phosphate Buffered Saline) will be collected under a laminar flow hood using aseptic technique and placed into a sterile amber glass serum bottle. The vehicle sample will be stored refrigerated (2-8°C) until shipped on ice packs to the address below via overnight, weekday delivery for possible analysis.

Dosing Formulation Sampling Procedure: Any remaining dosing formulation will be retained following dosing and stored refrigerated (2-8°C) until shipped on ice packs to the address below via overnight, weekday delivery for possible analysis.

Primary Contact for Shipment of Vehicle Sample and Remaining Dosing Formulation
DuPont Haskell Global Centers for Health and Environmental Sciences Attn: Michael Mawn, Ph.D. Stine-Haskell Research Center 1090 Elkton Road Newark, DE 19714 Telephone: (302) 451-3365 Telefax: (302) 451-3571 E-mail: michael.p.mawn@usa.dupont.com

Title of Study: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose
MPI Research Study Number: 125-099
Sponsor Study Number: WR 17751, SC 1579

Analysis of the remaining dosing formulation may be conducted by the Sponsor or a Sponsor-designated laboratory at the discretion of the Sponsor and the results will not be included as an appendix to the final report issued by MPI Research. The Sponsor and/or Sponsor-designated laboratory will be responsible for the conduct, reporting, and any regulatory requirements for these analyses.

Test Article Preparation Procedure: Standard laboratory procedures will be used. Specific procedures will be documented in the study data. Each test article will be used as received and no adjustment will be made for purity, salt correction, etc.

The test article will be provided as a concentrated solution that will be diluted with an appropriate volume of vehicle (sterile Phosphate Buffered Saline) to achieve the desired concentration. The procedure outlined below will be used as a guide to prepare the final dosing formulation:

1. Calculate the volume of vehicle required to add to the concentrated test article solution to achieve the required concentration.
2. Use a syringe to measure the vehicle at approximately 90% of the volume calculated in Step 1.
3. Measure the required volume of the concentrated test article solution and transfer into a clean glass beaker.
4. Stir the contents of the beaker using a magnetic stir bar and a stir plate and add the vehicle measured in Step 2 to the beaker.
5. Measure and record the initial pH.
6. While stirring, adjust the pH to 7.6 (±0.1) using an appropriate concentration of HCl or NaOH.
7. Transfer the solution into a graduated cylinder.
8. Thoroughly rinse the beaker with vehicle and transfer the rinse into the graduated cylinder.
9. Add vehicle to the graduated cylinder to yield the required volume of prepared dosing formulation.
10. Thoroughly mix the contents of the cylinder and filter the solution (under a laminar flow hood) through a 0.2 µm or 0.22 µm syringe filter (or vacuum filtration unit) into an appropriate number of sterile amber glass serum bottles prior to dosing.

Unless indicated otherwise, any remaining/unused test article(s) will be shipped to:

Primary Contact for Shipment of Remaining/Unused Test Article(s)	
DuPont Haskell Global Centers for Health and Environmental Sciences Attn: Michael Mawn, Ph.D. Stine-Haskell Research Center 1090 Elkton Road Newark, DE 19714	
Telephone: Telefax: E-mail:	(302) 451-3365 (302) 451-3571 michael.p.mawn@usa.dupont.com

Approval:

Sponsor Signature: Shawn A Gannon Date: 29-July-2006
Shawn A. Gannon, B.S.

APPROVED

Title of Study: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose
MPI Research Study Number: 125-099
Sponsor Study Number: WR 17751, SC 1579

Amendments/Changes in Protocol and Impact on Study:
The following details are added to the protocol and represent the standard study conditions/methods:

1. **Source of monkeys:** Animals used on study will be transferred from an MPI Research stock colony of male and/or female cynomolgus monkeys set aside specifically for use on PK studies. Original source/health records are on file at MPI Research. Each animal will be assigned an animal number to be used in Provantis™ and will be implanted with a microchip bearing a unique identification number. Each animal will have a permanent tattoo of a vendor animal number on the chest. The individual animal number, implant number, and the MPI Research study number will comprise a unique identification for each animal. The current state of scientific knowledge does not provide acceptable alternatives, *in vitro* or otherwise, to the use of live animals to accomplish the purpose of this study.

2. **Justification of Test System:** Although the beagle is the usual non-rodent model[1] used for evaluating the toxicity of various test articles and for which there is a large historical database, the monkey was selected specifically for use in this study by the Sponsor because:

   ☐ i) Data collected during the course of this study may be used to evaluate the pharmacokinetics/ bioavailability of the test article(s) in monkeys when compared to the beagle in order to determine which species would be the most suitable for future preclinical studies required by applicable regulatory agencies.

   ☐ ii) The Sponsor currently maintains a colony of non-naïve monkeys at MPI Research. In general, due to their longer lifespan and slower growth rate, maintaining and re-using monkeys in a stock colony may ultimately contribute to a reduction in the total number of animals required for the completion of the Sponsor's discovery/preclinical program(s).

   ☐ iii) The monkey is expected to be more tolerant of the route of administration or vehicle required for this study.

   ☒ iv) Sponsor-supplied justification: There are significant differences in the rate and manner of elimination of this compound in rodent species (rat and mouse) and between sexes. The goal of this study is to determine which rodent model is more relevant to primates.

3. **Numbers and body weight range of monkeys:** A total of 6 non-naïve cynomolgus monkeys (3 males and 3 females) will be initially assigned to study. Approximate weight range of 2.3-6 kg (young adults). Age (when available) will be maintained in the stock colony records. Actual weights of the animals will be documented in the data. After study termination, monkeys will be returned to the stock colony and, after a washout period of at least 1 week, may be utilized on another study. This study was designed to use the fewest number of animals possible, consistent with the objective of the study, the scientific needs of the Sponsor and contemporary scientific standards.

4. **Acclimation/selection/randomization:** All animals will have been previously acclimated at MPI Research. Only healthy animals will be selected for study. No randomization is necessary.

[1] Guidance for Industry, Investigators, and Reviewers: Exploratory IND Studies, U.S. F.D.A. Center for Drug Evaluation and Research (CDER), January 2006.

Title of Study: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose
MPI Research Study Number: 125-099
Sponsor Study Number: WR 17751, SC 1579

5. **Husbandry**: Due to the necessity to minimize potential disease transmission during quarantine and for rigorous monitoring during testing, all monkeys will be housed individually in stainless steel cages. The monkeys will be provided environmental enrichment during the quarantine and the study according to an MPI Research SOP. Fluorescent lighting will be provided via an automatic timer for approximately 12 hours per day. On occasion, the dark cycle may be briefly interrupted to allow for study functions that occur during the 12-hour dark cycle. Food and water will be available *ad libitum* except during fasting periods (when food only will be withheld) and during chair-restraint (when both food and water will be withheld). Lab Diet® Certified Primate Diet #5048 (PMI Nutrition International, Inc.) will be provided to all animals twice daily except during fasting periods. In addition, other certified enrichment foods will be offered during the study according to an MPI Research SOP and fresh fruits and vegetables may be offered during the acclimation period. These extra offerings will be documented in the study records. Temperature and humidity will be monitored and recorded daily and maintained to the maximum extent possible between 64 to 84°F and 30 to 70%, respectively. Routine feed/water analysis results are kept on file at MPI Research; no contaminants are likely to be present in food or water which would affect the outcome of the study.

6. **Test article and dosing preparation analyses:** The Sponsor has assumed responsibility for documenting the characteristics and results of analysis of the bulk test article(s) as well as the homogeneity/stability/concentration of the dosing formulation(s).

7. **Dosing preparation and administration:** Non-sterile dosing formulations should be continuously stirred until picked up for dosing and should continue to be stirred in the animal room throughout dosing administration. Prepared dosing formulations should be administered as soon as possible after preparation is complete. Prior to dosing, any non-sterile dosing formulations for injection (IV, SC, IM, IP) will be filtered through a 0.2 or 0.22 µm syringe filter (or vacuum filtration unit) into a necessary number of sterile amber glass serum bottles.

8. **Body weights:** Bodyweights will be collected on the day of dosing or the day before dosing for each dose.

9. **Fasting period before each dose (if applicable):** Animals will be fasted overnight prior to dosing and food will be withheld during the first 4 hours of blood collection (food will be returned within 30 minutes following collection of the last blood sample at the 4 hour time point, where applicable). Total fasting time will not exceed 24 hours. Animals will not be fasted more than three times during each study week and will not be fasted on consecutive days unless they have been fed according to the normal feeding schedule for at least 8 hours between each fasting period. Water will be withheld during chair restraint only (if applicable).

10. **Catheterizations:** The arm and/or leg vein(s) may be catheterized for blood sampling and for intravenous dosing using standard procedures. If a catheter is used for dosing, subsequent samples will be collected from a separate catheter placed at a different site or from the femoral vein/artery.

11. **Chair restraint**: Previously-acclimated animals may be restrained (if necessary) for a maximum of 3 hours for dosing and subsequent blood collection. At the end of restraint, catheters will be removed and monkeys will be returned to their cages and all remaining blood samples will be collected from the femoral vein/artery.

Title of Study: Collection of Plasma Samples for Determination of the Pharmacokinetics of FRD-902 in Male and Female Cynomolgus Monkeys Following Administration of a Single Intravenous Dose
MPI Research Study Number: 125-099
Sponsor Study Number: WR 17751, SC 1579

12. **Observations:** Twice-daily cageside observations for clinical signs/morbidity/mortality /injury/availability of food and water. Although no scheduled detailed clinical examinations will be conducted, positive clinical signs will be recorded at unscheduled intervals if observed, and the persistence of each sign will be recorded at subsequent collection intervals until the sign is no longer present or the Study Director instructs otherwise.

13. **Blood volume:** Total maximum volume of blood collected over a 2 week period must not exceed 10 mL/kg.

14. **Dead animals:** If any animals are found dead or are euthanized *in extremis* (per applicable MPI Research Standard Operating Procedures), an attempt will be made to contact the Sponsor representative/designee as soon as possible. A necropsy will be conducted to determine the possible cause of death and any abnormal macroscopic observations will be recorded. Necropsies will be done at additional cost. All carcasses will be discarded, unless otherwise specified by the Sponsor.

15. **Method of euthanasia (if necessary):** Per MPI Research Standard Operating Procedures, euthanasia will be by sodium pentobarbital solution administration, under ketamine sedation (if necessary), followed by an MPI Research SOP approved method to assure death, e.g. exsanguination.

16. **Statistical analysis:** No analyses of in-life data due to the small numbers of animals at each time point and the absence of a control group. The Sponsor will select any methods of statistical analysis for bioanalytical results.

Study Comments and Observations:

APPROVED

Appendix B
Primate In-Life Report

MPI RESEARCH

COLLECTION OF PLASMA SAMPLES FOR DETERMINATION OF THE PHARMACOKINETICS OF FRD-902 IN MALE AND FEMALE CYNOMOLGUS MONKEYS FOLLOWING ADMINISTRATION OF A SINGLE INTRAVENOUS DOSE

TEST ARTICLE:	FRD-902
TESTING FACILITY:	MPI Research, Inc. 54943 North Main Street Mattawan, Michigan 49071-9399
STUDY NUMBER:	125-099
PROTOCOL APPROVED BY SPONSOR:	July 29, 2008
STUDY INITIATION DATE (Protocol Signed by Study Director):	July 29, 2008
EXPERIMENTAL START DATE:	August 13, 2008
EXPERIMENTAL TERMINATION DATE:	September 14, 2008
DRAFT REPORT MAIL DATE:	September 30, 2008
STUDY DIRECTOR:	Travis L. Devlin, M.S., L.A.T.
SPONSOR:	DuPont Haskell Global Centers for Health and Environmental Sciences Stine-Haskell Research Center 1090 Elkton Road Newark, Delaware 19714
SPONSOR STUDY NUMBER:	WR 17751, SC 1579
SPONSOR REPRESENTATIVE:	Shawn A. Gannon, B.S.
DATE OF STUDY COMPLETION:	October 9, 2008

SIGNATURE

This report is being submitted by the following personnel.

Travis L. Devlin, M.S., L.A.T.
Study Director

10-9-08
Date

125-099

MATERIALS AND METHODS

Test Article Preparation

Standard laboratory procedures were used and no problems were encountered. The test article was received from the Sponsor at a concentrated solution (826 mg/mL) and was diluted with the required amount of vehicle to achieve the desired concentration. The pH of the dosing formulation was adjusted to 7.63 using 0.1N and 1N HCl (hydrochloric acid). Prior to dosing, the formulation was filtered through a PALL Acrodisc® syringe filter with a 0.2 µm HT Tuffryn membrane. The final formulation appeared to be a clear, colorless solution.

FRD-902	
Initial Concentration:	826 mg/mL
Volume of Concentrated Solution:	0.363 mL
Vehicle:	Phosphate Buffered Saline (PBS)
Volume of Final Preparation:	60 mL
Completion Time of Preparation:	7:56 A.M. on the day of dosing

Dosing Formulation Sampling Procedure and Disposition

A single sample (10 mL) of the vehicle was collected prior to test article preparation. The vehicle sample and the remaining dosing formulation were stored refrigerated until shipped on ice packs to the Sponsor for possible analysis. The remaining test article was stored at room temperature and desiccated until shipped under ambient condition to the Sponsor.

Dose Administration

On August 12, 2008, six non-naïve cynomolgus monkeys were transferred from an MPI Research stock colony and placed on study. The animals were fasted overnight prior to dosing and food was withheld through the first 4 hours of blood sample collection.

The test article, FRD-902, was administered via a single intravenous (IV) bolus injection at a dose volume of 2 mL/kg. Following administration the catheter was flushed with 1.0 mL of sterile 0.9% Sodium Chloride for Injection, USP. Dose administration information is presented in the following table.

125-099

FRD-902						
Animal Number	Group Number	Sex	Body Weight (kg)	Dose Concentration (mg/mL)	Dose Level (mg/kg)	Dose Volume (mL)
901	1	Male	2.42	5	10	4.8
902	1	Male	2.64	5	10	5.3
903	1	Male	2.27	5	10	4.5
904	1	Female	3.33	5	10	6.7
905	1	Female	3.36	5	10	6.7
906	1	Female	3.69	5	10	7.4

Blood Sample Collection and Analysis

Blood samples (approximately 0.5 mL) were collected from the femoral vessels and placed on ice. Samples were collected into tubes containing K_2EDTA. Sample collection information is presented in the following tables.

125-099

Plasma Sample Collection Treatment - FRD-902						
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample Time	Relative Sample Time	Actual Sample Time
901	1	Male	8:30 A.M.	Predose	-45 m	7:45 A.M.
				5 m	5 m	8:35 A.M.
				10 m	10 m	8:40 A.M.
				15 m	15 m	8:45 A.M.
				30 m	30 m	9:00 A.M.
				1 h	**1 h 1 m**	**9:31 A.M. (H)**
				2 h	2 h	10:30 A.M.
				4 h	4 h	12:30 P.M.
				8 h	8 h	4:30 P.M.
				12 h	12 h	8:30 P.M.
				24 h	24 h	8:30 A.M.
902	1	Male	8:32 A.M.	Predose	-45 m	7:47 A.M.
				5 m	5 m	8:37 A.M.
				10 m	10 m	8:42 A.M.
				15 m	15 m	8:47 A.M.
				30 m	30 m	9:02 A.M.
				1 h	1 h	9:32 A.M.
				2 h	2 h	10:32 A.M.
				4 h	4 h	12:32 P.M.
				8 h	8 h	4:32 P.M.
				12 h	12 h	8:32 P.M.
				24 h	24 h	8:32 A.M.
903	1	Male	8:34 A.M.	Predose	-45 m	7:49 A.M.
				5 m	5 m	8:39 A.M.
				10 m	10 m	8:44 A.M.
				15 m	15 m	8:49 A.M.
				30 m	**32 m**	**9:06 A.M. (H,C)**
				1 h	1 h	9:34 A.M.
				2 h	2 h	10:34 A.M.
				4 h	4 h	12:34 P.M.
				8 h	8 h	4:34 P.M.
				12 h	12 h	8:34 P.M.
				24 h	24 h	8:34 A.M.
m - minute h - hour			**Bold** - Late sample **H** - Hemolyzed sample **C** - Clotted sample			

125-099

Plasma Sample Collection Treatment - FRD-902						
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample Time	Relative Sample Time	Actual Sample Time
904	1	Female	8:36 A.M.	Predose	-45 m	7:51 A.M.
				5 m	5 m	8:41 A.M.
				10 m	10 m	8:46 A.M.
				15 m	15 m	8:51 A.M.
				30 m	30 m	9:06 A.M.
				1 h	1 h	9:36 A.M.
				2 h	**2 h 1 m**	**10:37 A.M.**
				4 h	4 h	12:36 P.M.
				8 h	8 h	4:36 P.M.
				12 h	12 h	8:36 P.M.
				24 h	24 h	8:36 A.M. (**C**)
905	1	Female	8:38 A.M.	Predose	-43 m	7:55 A.M.
				5 m	5 m	8:43 A.M.
				10 m	10 m	8:48 A.M.
				15 m	15 m	8:53 A.M.
				30 m	30 m	9:08 A.M.
				1 h	1 h	9:38 A.M.
				2 h	**2 h 1 m**	**10:39 A.M.**
				4 h	**4 h 1 m**	**12:39 P.M. (H,C)**
				8 h	**8 h 2 m**	**4:40 P.M.**
				12 h	12 h	8:38 P.M.
				24 h	24 h	8:38 A.M.
906	1	Female	8:40 A.M.	Predose	-45 m	8:05 A.M.
				5 m	5 m	8:45 A.M.
				10 m	10 m	8:50 A.M.
				15 m	15 m	8:55 A.M.
				30 m	30 m	9:10 A.M.
				1 h	1 h	9:40 A.M.
				2 h	2 h	10:40 A.M.
				4 h	**4 h 1 m**	**12:41 P.M.**
				8 h	**8 h 2 m**	**4:42 P.M.**
				12 h	12 h	8:40 P.M.
				24 h	24 h	8:40 A.M.
m - minute h - hour			**Bold** - Late sample **H** - Hemolyzed sample **C** - Clotted sample			

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 3	9:19 A.M.
902	1	Male	8:32 A.M.	Day 3	9:22 A.M.
903	1	Male	8:34 A.M.	Day 3	9:25 A.M.
904	1	Female	8:36 A.M.	Day 3	9:30 A.M.
905	1	Female	8:38 A.M.	Day 3	9:33 A.M.
906	1	Female	8:40 A.M.	Day 3	9:36 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 4	9:21 A.M.
902	1	Male	8:32 A.M.	Day 4	9:24 A.M.
903	1	Male	8:34 A.M.	Day 4	9:26 A.M.
904	1	Female	8:36 A.M.	Day 4	9:28 A.M.
905	1	Female	8:38 A.M.	Day 4	9:31 A.M.
906	1	Female	8:40 A.M.	Day 4	9:35 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 5	10:38 A.M.
902	1	Male	8:32 A.M.	Day 5	10:39 A.M.
903	1	Male	8:34 A.M.	Day 5	10:41 A.M.
904	1	Female	8:36 A.M.	Day 5	10:43 A.M.
905	1	Female	8:38 A.M.	Day 5	10:45 A.M.
906	1	Female	8:40 A.M.	Day 5	10:48 A.M.

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 6	1:07 P.M.
902	1	Male	8:32 A.M.	Day 6	1:11 P.M.
903	1	Male	8:34 A.M.	Day 6	1:13 P.M.
904	1	Female	8:36 A.M.	Day 6	1:15 P.M.
905	1	Female	8:38 A.M.	Day 6	1:18 P.M.
906	1	Female	8:40 A.M.	Day 6	1:22 P.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 7	9:44 A.M.
902	1	Male	8:32 A.M.	Day 7	9:47 A.M.
903	1	Male	8:34 A.M.	Day 7	9:49 A.M.
904	1	Female	8:36 A.M.	Day 7	9:50 A.M.
905	1	Female	8:38 A.M.	Day 7	9:52 A.M.
906	1	Female	8:40 A.M.	Day 7	9:54 A.M.

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 8	10:26 A.M.
902	1	Male	8:32 A.M.	Day 8	10:28 A.M.
903	1	Male	8:34 A.M.	Day 8	10:30 A.M. (**H,C**)
904	1	Female	8:36 A.M.	Day 8	10:32 A.M.
905	1	Female	8:38 A.M.	Day 8	10:34 A.M.
906	1	Female	8:40 A.M.	Day 8	10:35 A.M.
H - Hemolyzed sample **C** - Clotted sample					

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 9	7:25 A.M.
902	1	Male	8:32 A.M.	Day 9	7:27 A.M.
903	1	Male	8:34 A.M.	Day 9	7:29 A.M.
904	1	Female	8:36 A.M.	Day 9	7:31 A.M.
905	1	Female	8:38 A.M.	Day 9	7:33 A.M.
906	1	Female	8:40 A.M.	Day 9	7:35 A.M.

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 10	7:30 A.M.
902	1	Male	8:32 A.M.	Day 10	7:33 A.M.
903	1	Male	8:34 A.M.	Day 10	7:36 A.M.
904	1	Female	8:36 A.M.	Day 10	7:38 A.M.
905	1	Female	8:38 A.M.	Day 10	7:41 A.M.
906	1	Female	8:40 A.M.	Day 10	7:45 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 11	10:41 A.M.
902	1	Male	8:32 A.M.	Day 11	10:42 A.M.
903	1	Male	8:34 A.M.	Day 11	10:43 A.M.
904	1	Female	8:36 A.M.	Day 11	10:45 A.M.
905	1	Female	8:38 A.M.	Day 11	10:46 A.M.
906	1	Female	8:40 A.M.	Day 11	10:48 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 12	10:42 A.M.
902	1	Male	8:32 A.M.	Day 12	10:43 A.M.
903	1	Male	8:34 A.M.	Day 12	10:45 A.M.
904	1	Female	8:36 A.M.	Day 12	10:46 A.M.
905	1	Female	8:38 A.M.	Day 12	10:47 A.M.
906	1	Female	8:40 A.M.	Day 12	10:49 A.M.

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 13	8:03 A.M.
902	1	Male	8:32 A.M.	Day 13	8:07 A.M.
903	1	Male	8:34 A.M.	Day 13	8:11 A.M.
904	1	Female	8:36 A.M.	Day 13	8:14 A.M.
905	1	Female	8:38 A.M.	Day 13	8:18 A.M.
906	1	Female	8:40 A.M.	Day 13	8:26 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 14	7:34 A.M.
902	1	Male	8:32 A.M.	Day 14	7:37 A.M.
903	1	Male	8:34 A.M.	Day 14	7:40 A.M.
904	1	Female	8:36 A.M.	Day 14	7:41 A.M.
905	1	Female	8:38 A.M.	Day 14	7:46 A.M.
906	1	Female	8:40 A.M.	Day 14	7:50 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 15	7:32 A.M.
902	1	Male	8:32 A.M.	Day 15	7:34 A.M.
903	1	Male	8:34 A.M.	Day 15	7:36 A.M.
904	1	Female	8:36 A.M.	Day 15	7:38 A.M.
905	1	Female	8:38 A.M.	Day 15	7:41 A.M.
906	1	Female	8:40 A.M.	Day 15	7:43 A.M.

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 16	7:51 A.M.
902	1	Male	8:32 A.M.	Day 16	7:53 A.M. (**H**)
903	1	Male	8:34 A.M.	Day 16	7:55 A.M.
904	1	Female	8:36 A.M.	Day 16	7:57 A.M.
905	1	Female	8:38 A.M.	Day 16	8:00 A.M.
906	1	Female	8:40 A.M.	Day 16	8:04 A.M.
H - Hemolyzed sample					

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 17	7:20 A.M.
902	1	Male	8:32 A.M.	Day 17	7:22 A.M.
903	1	Male	8:34 A.M.	Day 17	7:25 A.M.
904	1	Female	8:36 A.M.	Day 17	7:26 A.M. (**C**)
905	1	Female	8:38 A.M.	Day 17	7:28 A.M.
906	1	Female	8:40 A.M.	Day 17	7:31 A.M.
C - Clotted sample					

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 18	9:52 A.M.
902	1	Male	8:32 A.M.	Day 18	9:55 A.M.
903	1	Male	8:34 A.M.	Day 18	9:57 A.M.
904	1	Female	8:36 A.M.	Day 18	9:59 A.M.
905	1	Female	8:38 A.M.	Day 18	10:02 A.M.
906	1	Female	8:40 A.M.	Day 18	10:06 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 19	9:16 A.M.
902	1	Male	8:32 A.M.	Day 19	9:17 A.M.
903	1	Male	8:34 A.M.	Day 19	9:19 A.M.
904	1	Female	8:36 A.M.	Day 19	9:20 A.M.
905	1	Female	8:38 A.M.	Day 19	9:21 A.M.
906	1	Female	8:40 A.M.	Day 19	9:23 A.M.

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 20	10:46 A.M.
902	1	Male	8:32 A.M.	Day 20	10:47 A.M.
903	1	Male	8:34 A.M.	Day 20	10:49 A.M.
904	1	Female	8:36 A.M.	Day 20	10:51 A.M.
905	1	Female	8:38 A.M.	Day 20	10:52 A.M.
906	1	Female	8:40 A.M.	Day 20	10:54 A.M.

125-099

Plasma Sample Collection Treatment - FRD-902					
Animal Number	Group Number	Sex	Dose Time, 8/13/08	Nominal Sample	Sample Time
901	1	Male	8:30 A.M.	Day 21	9:07 A.M.
902	1	Male	8:32 A.M.	Day 21	9:08 A.M.
903	1	Male	8:34 A.M.	Day 21	9:11 A.M.
904	1	Female	8:36 A.M.	Day 21	9:13 A.M.
905	1	Female	8:38 A.M.	Day 21	9:15 A.M.
906	1	Female	8:40 A.M.	Day 21	9:17 A.M.

The samples were centrifuged at 2 to 7°C following completion of sample collection at each interval. The resulting plasma was separated and stored frozen at approximately -70°C until shipped on dry ice to the Sponsor for analysis.

Animal and Data Disposition

Animal Final Disposition

The animals were transferred to an MPI Research stock colony following the last blood sample collection interval.

Data Disposition

All raw data, documentation, records, protocol, and the final report generated as a result of this study will be retained at MPI Research, Inc., or an approved archive facility contracted by MPI Research, Inc., for a period of 1 year following completion of the study (final report issue date). Retention of materials after the time stated above will be subject to future contractual agreements.

125-099

RESULTS

Clinical Observations

Predose Observations

Animal number 904 was observed with watery feces prior to dosing. No additional findings were recorded predose.

Postdose Observations

Positive clinical findings observed during the coarse of the study are presented in the following table.

Positive Clinical Findings[a]

Animal Number	Group Number	Sex	Observation	Interval
901	1	Male	Abdomen distended	Day 1 (8 h 14 m postdose)
			Abrasion(s), face	Days 4 and 6
			Abrasion(s), lower jaw	Days 3, 5, and 7
			Feces soft	Days 5-6
			Feces watery	Days 3-4
902	1	Male	Abdomen distended	Day 1 (8 h 13 m postdose) Day 1 (12 h postdose) Days 2-3, 7, and 8
			Emesis	Day 12
			Feces soft	Day 9
903	1	Male	Abdomen distended	Day 1 (8 h 11 m postdose) Day 1 (12 h postdose)
904	1	Female	Feces soft	Days 9 and 13
			Feces watery	Day 2
906	1	Female	Abdomen distended	Day 1 (8 h 5 m postdose) Day 1 (12 h 1 m postdose) Days 2-3, 6-12, 20-21
			Feces soft	Day 9
			Skin cold to touch	Day 1 (12 h 1 m postdose)

m - minute
h - hour

[a]Negative findings are documented in the study file.

Following collection of the final blood sample on Day 21, animal number 906 was placed under veterinary consultation for daily monitoring for approximately 4 days. These findings are not reported but are maintained in the study file.

125-099

DEVIATIONS

This study was conducted in accordance with the protocol, with the exception of the following deviations:

On Day 1, the 30 minute postdose blood sample for animal number 903, the 4 hour postdose blood sample for animal number 905, and the 24 hour postdose blood sample for animal number 904 were each clotted, resulting in serum instead of plasma.

On Day 8, the blood sample collected for animal number 903 was clotted, resulting in serum instead of plasma.

On Day 17, the blood sample collected for animal number 904 was clotted, resulting in serum instead of plasma.

On three occasions, the temperature of the animal room fell outside of the protocol required range of 64 to 84ºC

In the opinion of the Study Director, these deviations did not affect the quality or integrity of the study.

125-099

Appendix C
Individual LC/MS Rat Plasma Sample Results

Individual LC/MS plasma sample results.

	FRD-902 plasma concentration for the specified timepoint (ng/mL) for 10 mg/kg dose level														
Animal Number	Predose	5 min	15 min	30 min	1 Hour	2 Hour	4 Hour	8 Hour	12 Hour	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour	168 Hour
Rat 1 Male	28.6	53200	52900	48200	43000	41500	22600	5420	2750	562	75.5	51.5	87.6	17.8	65.8	27.2
Rat 2 Male	2.32	123000	62100	53600	53300	44900	23000	8580	3300	746	104	38.9	57.2	39.3	26.0	20.8
Rat 3 Male	<1.0	60200	54300	55700	40900	42100	17200	6110	5150	1020	44.6	42.4	24.1	66.3	25.7	17.9
Rat 4 Female	20.8	54300	32100	20000	6290	2670	404	510	95.2	3.66	8.00	14.3	7.78	<1.0	2.42	2.1
Rat 5 Female	<1.0	73800	32700	20000	5640	2130	321	253	73.3	12.1	6.24	14.4	5.99	1.97	1.84	<1.0
Rat 6 Female	1.16	60500	30900	19300	7350	1430	284	44.1	57.2	5.65	32.9	8.49	2.80	3.02	2.27	2.05

	FRD-902 plasma concentration for the specified timepoint (ng/mL) for 50 mg/kg dose level														
Animal Number	Predose	5 min	15 min	30 min	1 Hour	2 Hour	4 Hour	8 Hour	12 Hour	24 Hour	48 Hour	72 Hour	96 Hour	120 Hour	144 Hour	168 Hour
Rat 1 Male	8.72	354000	296000	273000	206000	142000	44200	10500	7820	1290	1350	117	94.4	94.5	42.1	67.1
Rat 2 Male	1.54	371000	358000	322000	267000	145000	83800	35000	14700	3650	887	122	196	55.3	87.3	69.1
Rat 3 Male	15.8	408000	381000	261000	227000	200000	48800	12800	4250	713	824	202	91.5	185	139	90.3
Rat 4 Female	22.2	383000	274000	166000	93900	30200	4780	498	349	129	38.5	147	12.1	19.1	34.1	28.7
Rat 5 Female	19.3	308000	269000	201500	116000	39400	3860	307	336	57.6	90.3	109	51.7	58.5	64.7	21.3
Rat 6 Female	<1.0	398000	282000	204000	117000	32300	4340	1720	1370	46.4	122.0	45.9	47.8	57.9	55.7	27.6

Appendix D
Individual LC/MS Primate Plasma Sample Results

Individual LC/MS/MS Plasma Sample Results

	FRD-902 Plasma Concentration in ng/mL					
	Male			Female		
Time Point	Animal 901	Animal 902	Animal 903	Animal 904	Animal 905	Animal 906
0	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
5 min	218000	189000	178000	225000	299000	143000
10 min	189000	184000	162000	202000	183000	253000
15 min	177000	164000	139000	174000	164000	233000
30 min	136000	116000	68100	152000	126000	169000
1 Hr	94200	81400	61100	104000	84400	113000
2 Hr	51200	44400	36600	54800	43800	53600
4 Hr	20300	19000	10100	15600	17400	15500
8 Hr	5790	7980	2450	4520	5900	4330
12 Hr	1560	3410	883	1350	1690	1200
24 Hr	126	458	155	58.0	142	48.3
72 Hr	22.4	121	57.6	17.8	58.5	9.06
96 Hr	6.99	24.4	12.7	2.46	5.66	2.07
120 Hr	5.47	7.49	9.57	4.91	3.14	1.65
144 Hr	9.09	7.78	7.80	14.8	8.39	2.46
168 Hr	3.24	3.67	4.98	1.34	<1.00	<1.00
192 Hr	2.89	2.66	4.08	1.20	<1.00	<1.00
216 Hr	2.56	3.05	5.27	<1.00	<1.00	<1.00
240 Hr	2.69	3.38	6.70	1.17	<1.00	<1.00
264 Hr	4.05	5.90	3.62	3.00	3.04	3.67
288 Hr	2.74	5.09	2.56	1.29	<1.00	<1.00
312 Hr	2.17	2.29	3.24	<1.00	<1.00	<1.00
336 Hr	1.67	4.15	2.88	1.19	<1.00	<1.00
360 Hr	2.00	2.48	3.01	<1.00	1.79	<1.00
384 Hr	1.91	2.20	2.77	<1.00	1.99	<1.00
408 Hr	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
432 Hr	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
456 Hr	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
480 Hr	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
504 Hr	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00